Genomics for food biotechnology

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Genomics for food biotechnology: prospects of the use of high-throughput technologies for the improvement of food microorganisms

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Functional genomics is currently the most effective approach for increasing the knowledge at the molecular level of metabolic and adaptive processes in whole cells. High-throughput technologies, such as DNA microarrays, and improved two-dimensional electrophoresis methods combined with tandem mass-spectroscopy, supported by bioinformatics, are useful tools for food biotechnology, which depends on detailed knowledge of the properties of food microbes (and pathogens) in their industrial, food and consumer environments. Genomics of food microbes, based on rapidly emerging genome sequence information, generates valuable knowledge that can be used for metabolic engineering, improving cell factories and development of novel preservation methods. Furthermore, pre- and probiotic studies, characterization of stress responses, studies of microbial ecology and, last but not least, development of novel risk assessment procedures will be facilitated.

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Introduction

Food biotechnology, apart from the direct use of plants and animals, is mainly concerned with the application of (live) food-grade microorganisms, such as yeasts, fungi, bacilli and lactic acid bacteria, in industrial processes. Major goals are enhanced production of ingredients and improved properties of starter cultures, for example, reproducible growth characteristics, increased flavour formation and proteolytic activities or better autolytic properties. Determination of the influence of food microbes on human health is also an important area, for example, in pre- and probiotics research [1]. On the other side, the prevention of food-spoilage and pathogenic microbes needs attention. The aims of food biotechnology are not only directed towards improving food production, but are also defined by consumer demands for safe, natural, fresh, tasteful and convenient products [2].

The research programs for improvement of industrial properties of microorganisms used for fermentation were initially focussed on strain selection after classical mutagenesis, later followed by more directed approaches using genetic engineering. The main drawbacks of these approaches are that they are time-consuming, side-effects occurring in the selected or constructed strains are hard to predict and assess, and the full range of engineering possibilities cannot be exploited, due to lack of knowledge of inter-related regulatory and metabolic processes going on in a cell. Furthermore, part of the consumer population has serious worries about the safety of these novel foods, and whether unwanted spread of genetically modified microorganisms in nature can be controlled. It is exactly in the last two fields that functional genomics can provide solutions. Several criteria and options for safe biotechnology and for risk-assessment procedures for the environmental application of microorganisms have recently been discussed [3].

Implementation of functional genomics programs on food microorganisms will allow us to reach various industrial goals within the coming decade. For example, to determine side-effects of genetic alterations on functionality in products, to create desired pleiotropic effects by specific regulatory mutations, to predict and improve stress responses, to develop novel antimicrobial systems, and to direct metabolic engineering efforts. Furthermore, it will enable the identification of novel (enzyme) activities, the performance of high-throughput mutation analyses in strains of interest and the development of fast identification methods for food-spoilage or pathogenic microorganisms. This review provides an overview of current developments in high-throughput technologies and discusses their usefulness for food-biotechnology.

Functional genomics with microorganisms

High-throughput technologies

The recent burst in genome sequences of food-related microorganisms [4•] (Table 1) has opened the way for functional genomics approaches, including transcriptome, proteome and metabolome analyses, as well as structural genomics. New improvements in large-scale sequencing methodologies are continuously being reported [5•], indicating that even faster accumulation of sequencing data is to be expected. It is already claimed by several companies and institutions that an average bacterial genome can be sequenced in just a couple of days, setting a trend for the future in which any project involving an industrial microorganism might start with determining its genome sequence. In order to efficiently exploit this information, novel high-throughput analytical methods are being developed, such as DNA microarrays (transcriptome) [6,7,8•] and improved 2D-electrophoresis methods combined with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopic analysis (proteome) [9••]. The huge number of experimental data generated are gathered into large databases, the interpretation of which greatly depends

Table 1

Overview of genome sequence information of food-related microorganisms.

Food-grade bacteria	
Bacillus subtilis	Published
Saccharomyces cerevisiae	Published
Lactococcus lactis	Finished (to be published)
Aspergillus nidulans	In progress
Lactobacillus acidophilus	In progress
Lactobacillus sp. (WCFS/Greenomics, the Netherlands)	In progress
**Streptococcus thermophilus*	In progress
Pathogens/spoilage	
Escherichia coli	Published
Helicobacter pylori	Published
Campylobacter jejuni	In progress
Clostridium acetobutylicum	In progress
Enterococcus faecalis	In progress
Listeria monocytogenes	In progress
Mycobacterium tubercolosis	In progress
Salmonella typhimurium	In progress
Shigella flexneri	In progress
Staphylococcus aureus	In progress
Streptococcus mutans	In progress
Streptococcus pneumoniae	In progress
Streptococcus pyogenes	In progress
Vibrio cholerae	In progress

See [4•]. *B Purnelle, D Prozzi, P Hols, Université Catholique de Louvin, Belgium.

on novel bioinformatics methodologies, which should enable linking of the databases and facilitate classification and interpretation of the results. Fast developments are also taking place in bioinformatics. Systems for cluster analysis and display of genome-wide expression patterns have been developed that provide information on the status of cellular processes and on the possible functions of genes without a known functionality [10•]. A nice overview of existing databases on microbial genomes, genomics and proteomics, including an extensive list of relevant websites is available [11]. Novel data-mining and -linking approaches get increasing attention, yielding methods for determining microbial genescapes. Examples are the determination of phyletic and functional patterns of open reading frame (ORF) distribution among prokaryotes, providing a quantitative analysis tool for comparative genomics [12].

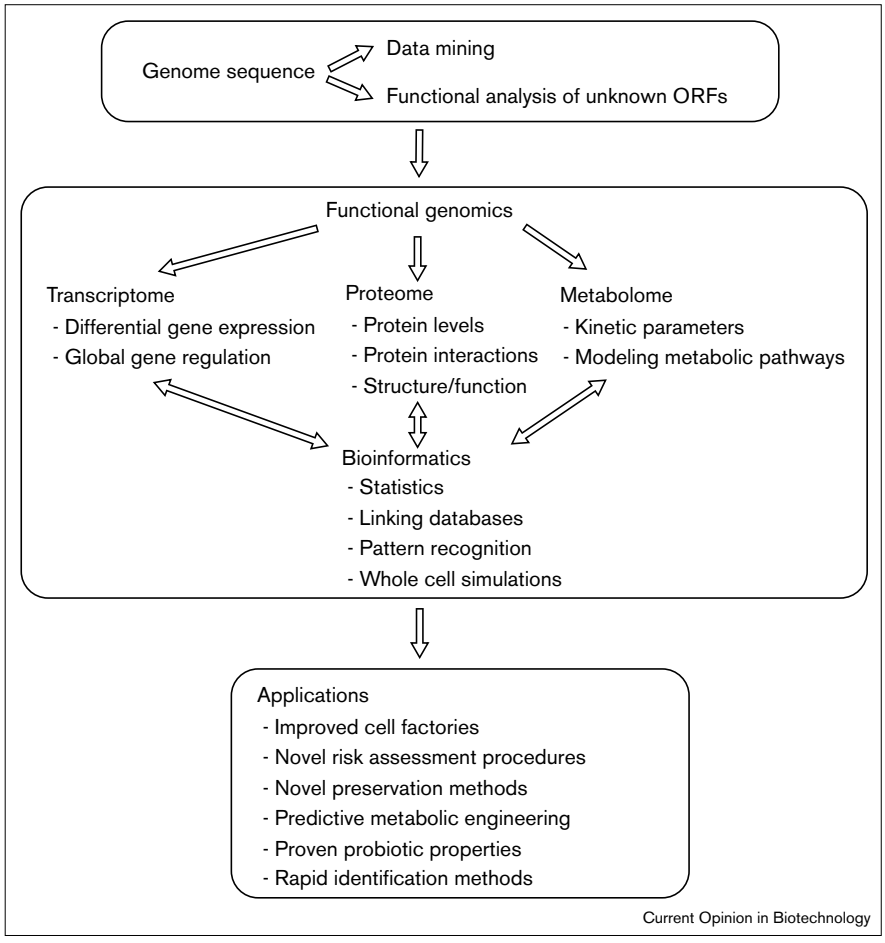
Differential gene expression

DNA microarrays provide a powerful tool for analyzing transcription profiles of whole genomes of any organism [6,7,8•]. A popular type of DNA chip is provided by Affymetrix (Santa Clara, CA), a company that synthesizes large arrays of oligonucleotides in micro-format on solid materials by photolithography. These arrays have already successfully been used for bacterial transcriptome analyses of 100 genes of *Streptococcus pneumoniae* and 106 genes of *Haemophilus influenzae*. It was found that the sensitivity of detection was very high, ranging between one and five transcripts per cell and that the data were in good agreement with conventional Northern blot results [13]. Another straightforward way of producing DNA microarrays is to spot amplicons of each ORF annotated in the genome sequence of interest on a defined support material, preferably glass-slides. Amplicons can conveniently be generated by PCR, and subsequently spotted onto glass slides. Over 100,000 spots per cm^2 can be accommodated on glass slides, providing sufficient combinatorial possibilities for bacterial genome applications. Fluorescently labeled cDNA is used for hybridization to the DNA arrays and the signals are detected by confocal laser scanning. The wild-type and mutant-strain cDNAs can be differentially labeled and used in one combined sample for hybridization, providing the attractive possibility of multiplexing. This will even allow the simultaneous data acquisition of several different cDNA samples, depending on the number of different fluorescent dyes that can be used. The data obtained provide information on differential gene expression. In essence, levels of all genes (mRNA converted into labeled cDNA) of the relevant microorganism are measured relative to a standard (e.g. a wild-type strain's expressed genes) by hybridizing the labeled cDNA to the whole cell genome displayed on the DNA microarray. This methodology is extremely useful for studying pleiotropic regulatory effects, for example, those involved in stress response, in complex regulatory cascades and in resistance mechanisms against antibiotics, antimicrobial peptides, phages and/or industrial stress conditions [6,7,8•,14–18].

Of course, specific DNA arrays can be developed for various other purposes, for example, rapid identification of pathogens and spoilage bacteria, mutation analyses, and for studying DNA–protein interactions. Furthermore, RNA of related or even less-related species can also be used for differential gene expression, using less stringent hybridization conditions, by means of virtual expression arrays [19]. These arrays are called virtual because the genome displayed is that of a different organism. Only part of the expressed genes under investigation will hybridize to those arrays, still providing valuable information. It is clear that many more genome sequences of food-microbes will become available in the near future, which creates attractive possibilities for studying these organisms in their industrial environments or in their host. On the side of pathogens and food-spoilage bacteria, a fast increase in sequencing data is also taking place (Table 1), creating exciting possibilities for the development of novel antimicrobial and antiviral strategies, and the study of their *in situ* activities, for example, in food products or in the mammalian gastro-intestinal tract. Finally, risk assessment procedures for using genetically modified organisms (GMOs) in food can be developed, applying transcript-imaging of various wild-type industrial strains and comparing the natural window of expression levels at a given time of growth to that of a particular GMO (P Renault *et al*., personal communication). In this way possible risk factors (i.e. proteins encoded by those genes that are expressed at a level outside the natural window) can be identified and then be analyzed further.

Figure 1

Functional genomics of food microorganisms.



Proteomics and structure–function relationships

The combination of transcriptome with proteome and metabolome research and the elucidation of structure–function relationships of biomolecules will eventually result in a true understanding of whole-cell functioning. Figure 1 gives an overview of approaches involved in functional genomics programs and their expected deliverables for food biotechnology. The proteome can be regarded as the expressed protein complement of a genome. Essentially, two types of proteomic research can be distinguished: first, expression proteomics, which aims at determining global changes in protein production levels; and second, cell-map proteomics, which systematically studies the protein–protein interactions through the isolation of protein complexes [9••]. For expression proteomics, 2D-gel electrophoresis is used for protein separation, while MALDI-TOF mass spectroscopy is applied for protein identification. Goals are to determine quantitative global changes in protein production in cells by image analysis. Assigning function to a detected protein requires a combinatorial approach, involving mutant analyses, application of (yeast) two hybrid systems, high-throughput protein structure determination and prediction, purification of protein complexes and subsequent mass-spectroscopic identification of the components, and bioinformatics, for example, to build virtual cells. Several pharmaceutical companies are heavily involved in proteomic research, with major goals to identify novel drug targets [20,21] for use in diagnostics and therapeutics.

Food biotechnology faces similar needs, such as determining the right targets for metabolic engineering, studying resistance mechanisms of food pathogens and identification of probiotic strains (e.g. those with good persistance in the gastro-intestinal tract while producing presumed health-promoting factors,) and *in situ* activities of lactic acid bacteria. Computational genomics should also be integrated with proteomics for the detection of functional and structural evolutionary relationships between proteins with only limited sequence similarities [22]. Established tools in life-sciences research, such as liquid chromatography and (capillary) electrophoresis, are now being examined for their potential to be used in small chip-based systems [23••]. These systems provide high-throughput possibilities for enzyme assays, restriction mapping of DNA, sequencing and immunological assays, thereby greatly speeding up research efforts because thousands of analyses

can be made in parallel. A second great advancement of chip-based systems is that they allow analytical chemistry to be performed in non-laboratory environments, which is an attractive perspective in food industry. Proteomics can be regarded as the intermediate step between transcriptome and metabolome [21]. Eventually, one would like to integrate all data on the transcriptome and proteome into a metabolic and regulatory model. Novel developments in this field are described in the following section.

Bioinformatics

An exciting development in bioinformatics is the building of an integrative model called E-CELL [24••], for simulating intracellular molecular processes to predict the dynamic behavior of cells, incorporating information on gene regulation, signaling and metabolism. It is equipped with graphical interfaces that allow observation and visualisation of interactions during cellular processes. The system gives the user the opportunity to define functions of proteins, macromolecular interactions, and characteristics of cellular metabolism as a set of reaction rules and eventually allows for *in silico* experimenting. The first constructed model was that of a hypothetical cell with only 127 genes sufficient for transcription, translation, energy production and phospholipid synthesis, which were mostly taken from the smallest known genome (i.e. that of *Mycoplasm genitalium*). This model is extremely useful for simulations of food microbes with relatively small genomes, such as those of lactic acid bacteria. The system can continuously be improved by adding novel experimental data, which are currently generated at a high pace.

Another interesting project is called 'the Virtual Cell' [25], which aims at modeling of cellular processes to be used as a tool for experimental biologists, for example, for metabolic engineering purposes. The input comes from users providing biochemical and electro-physiological data on any cell of interest. The powerful approach in functional genomics of using knowledge coming from metabolic engineering studies and gene–phenotype relationships for model building forms a major challenge in bioinformatics [26•]. Further developments in bioinformatics are ongoing, especially in the fields of genome mining, including functional analysis of genes with known and unknown function, linking databases with experimental data (transcriptome, proteome, metabolome, protein structure and function) and pattern recognition (see also Figure 1). An extensive and useful list of databases and Internet links is provided in a recent issue of *Nucleic Acids Research* [27•]. The '*Trends Guide to Bioinformatics*' provides an interesting overview of recent developments and opinions in bioinformatics [28].

Prospects and first examples of functional genomics with food microbes

Saccharomyces cerevisiae

Major biotechnological challenges in yeast research are the improvement of fermentative properties, osmotolerance and cryoresistance [29•]. Several genomics tools are already being applied in yeast research, of which the functional analysis program (e.g. EUROFAN), putting mutants through many specialized functional assays, is a very important one [30]. The use of DNA microarrays [31], metabolome analysis [32••] and the application of serial analysis of gene expression (SAGE), which is based on sequencing randomly concatenated transcript tags (e.g. to identify expressed small genes), as well as novel bioinformatics tools have also proven to be indispensable [32••,33].

Lactic acid bacteria and *Bacilli*

Recently, complete genome sequences of *Bacillus subtilis* [34] and *Lactococcus lactis* [35] became available, opening the way for functional genomics with these species of Gram-positive bacteria. For *B. subtilis* there are several initiatives in functional analysis to assess the function of all ORFs and to optimize secretion to obtain improved cell factories [36]. Furthermore, membrane-filter-based DNA microarrays for the whole genome are commercially available, and soon glass slides will also be prepared in our own lab for studies on complex gene regulation mechanisms and secretion processes. Exciting scientific developments, such as cytological methods to look at the cellular location of processes going on [37•] make genomics with *B. subtilis* even more effective. For *L. lactis* the situation is somewhat different as its complete genome sequence is expected to appear early 2000 in the public literature. However, in view of the excellent research tools (e.g. highly efficient controlled gene expression systems [38]) and detailed knowledge of environmental stress responses [39], proteolytic systems, gene regulation and metabolic pathways, and its frequent application in food biotechnology, it is expected that functional genomics of this organism will rapidly come of age [40]. Furthermore, *L. lactis* provides a model system for other lactic acid bacteria, which are currently being sequenced (i.e. *Lactobacillus acidophilus*, *Lactobacillus* sp. and *Streptococcus thermophilis* [Table 1]).

Pathogens and spoilage bacteria

In the field of food protection, it is of eminent importance to be able to identify and type pathogenic and spoilage microorganisms at an early stage. Plasmid typing, pulsed-field gel electrophoresis, ribotyping and random-amplified polymorphic DNA analyses are established methods for molecular typing [41]. With the ever-increasing availability of genome sequences of these microorganisms, however, genotypic methods that are amenable to automation will be developed. It can be foreseen that DNA microarrays with specific probes for thousands of different species or strains will be developed, enabling fast and reliable identification of microorganisms.

Another issue is the prevention of food spoilage and pathogens in food, relying on novel antimicrobial systems that are functional in food. Integrated databases describing pathway-based analyses of genomes of microorganisms and novel software tools aimed at the development of

antimicrobial agents in medicine [42], will also be valuable for the development of novel systems to fight food pathogens and spoilage organisms. A problem that might still persist is the development of resistance against antimicrobial substances in unwanted microorganisms in food. The mechanisms by which bacteria become resistant are still poorly understood, but use of DNA microarrays to analyse sensitive and resistant strains might identify the underlying metabolic and biosynthetic pathways, thereby defining the targets for effective food-grade antimicrobials.

An interesting concept for studying gene expression of prokaryotes for which classical genetic tools are not available (e.g. because they are hard to transform) involves making artificial chromosome libraries in *Escherichia coli*. A bacterial artificial chromosome (BAC) library of the spoilage bacterium *Bacillus cereus* was screened and a significant number of characteristic activities were detected, enabling functional genomics with these types of microorganisms [43].

Conclusions and future perspectives

Food biotechnology will benefit greatly from a functional genomics approach, which will create novel opportunities to ensure the safety of foods, to improve the quality of fermented products and to substantiate health claims related to the ingestion of specific microbes. Furthermore, industrial production of ingredients will be optimized by a better understanding of secretion processes, stress-responses and complex regulatory mechanisms. Exciting new technologies for life sciences are being developed with amazing speed. The gaps between transcriptome and proteome research are being bridged by recent breakthroughs, for example, the synthesis of double-stranded oligonucleotide arrays for parallel investigations of DNA–protein interactions [44•]. Advances in mass spectroscopy enable direct analysis of protein complexes, as a recent study on the *S. cerevisiae* ribosome showed. The method can identify more than 100 proteins in a single run, thus enabling the simultaneous analysis of all components of very large macromolecular complexes [45]. Undoubtedly, these and other novel methods in genomics will profoundly change the nature of food biotechnology in the next millennium.

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